

Fish hosts of the Carolina heelsplitter (*Lasmigona decorata*), a federally endangered freshwater mussel (Bivalvia: Unionidae)

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Abstract: Two laboratory trials were conducted to determine the required host fish for the Carolina heelsplitter (*Lasmigona decorata* (Lea, 1852)), an endangered freshwater mussel (Unionidae). The first trial used glochidia from a female collected from the Yadkin-Pee Dee River basin, and the second trial used the glochidia of an adult collected from the Catawba River basin. Two different techniques were utilized for glochidia extraction: flushing and serotonin-induced release. The first female tested (Yadkin-Pee Dee) packaged most of its glochidia attached to unfertilized eggs, and extraction of glochidia by flushing the marsupia with a syringe yielded few glochidia and caused extensive tearing of the gill tissue. In the second trial (Catawba) the female was immersed in 500 mg/L serotonin creatinine sulfate, and the glochidia were readily released without injury to the adult. Several species of minnows (Cyprinidae) from both basins served as hosts. Some sunfish species (Centrarchidae) supported transformation of a few juveniles, but differences in transformation success were observed between the two basins on these species.

Key words: Cyprinidae, glochidia, serotonin, conglutinate, Unionidae

Artificial propagation and culture has been identified as an important tool for conservation of native freshwater mussels (Unionidae) in the United States (National Native Mussel Conservation Committee 1998). The larval stage (glochidia) of most species of freshwater mussels must attach to the gills or fins of a fish to complete their metamorphosis into juveniles (Williams *et al.* 2008). Both male and female adult mussels and the appropriate fish hosts must be available for reproduction and the recruitment of new juveniles into the population. This obligate, parasitic relationship between mussel larvae and fish host can be a bottleneck to population expansion. As mussel populations decline, the probability of successful mating and subsequent population recruitment also declines (McLain and Ross 2005). Unionids reared in captivity can be used to augment declining populations, and moving this process to a controlled environment for artificial propagation can help bypass the bottleneck. Captive reared individuals may also serve as a resource for sentinel field or controlled laboratory studies and provide animals for educational exhibits.

One of the obstacles to the propagation of mussels native to the Atlantic slope is that the required host fishes are unknown for many species (Bogan 2002). Fish hosts can be determined in a variety of ways. Fish can be collected from the wild during the time of year they are most likely to be infected with glochidia from a specific species of mussel (Zale and Neves 1982a, 1982b). Fish gills and fins are then exam-

ined closely for glochidial attachment, and those glochidia are then identified by taking physical measurements of the shell (Surber 1912) or through molecular techniques (White *et al.* 1994, Gerke and Tiedemann 2001, Kneeland and Rhymer 2007). While this method provides documentation of attachment to a specific species in the wild, attachment to a fish does not necessarily reflect successful transformation. Mussels commonly have some host species that yield only a few transformed individuals, in contrast to other more effective fish-hosts that successfully support the transformation of large numbers of glochidia to juvenile mussels (Khym and Layzer 2000, Rogers *et al.* 2001, Van Snik Gray *et al.* 2002). Wild-caught fish infected with glochidia can also be held in aquaria until transformation is completed (Surber 1913, Coker *et al.* 1921). When fish are held individually in aquaria or as a species cohort, the ability of a specific species to support transformation is confirmed. However, both field-based methods may require extensive fish sampling to find the glochidia of a rare mussel species because the percentage of fish infected with glochidia approaches 0% at sites with low mussel densities (McLain and Ross 2005).

Laboratory-based host determinations are conducted by acquiring non-infested fish that co-occur with a mussel species, exposing them to glochidia obtained from gravid females, and harvesting juveniles after transformation is complete (Zale and Neves 1982a). After transformation, glochidia that attach to individually housed cohorts or

individuals can be collected, and the relative ability of different species of fish to support transformation can be compared. Laboratory-based infestations may not necessarily reflect field conditions, but they confirm transformation—a necessary step before proceeding with propagation.

The Carolina heelsplitter (*Lasmigona decorata* (Lea, 1852)) was historically found from small streams to large rivers and even millponds in the Pee Dee, Catawba, Savannah, and the Saluda River systems (Bogan 2008). Now federally endangered, it has been restricted to a few fragmented populations, primarily in headwater streams, in North and South Carolina (U.S. Fish and Wildlife Service 1996). The remaining populations in North Carolina and some in South Carolina lie in head-water streams near the rapidly expanding Charlotte, North Carolina metropolitan area. As outlying areas have been developed, changing land-use practices have altered stream habitat and degraded water quality. These watershed practices and a decade long drought have imperiled *L. decorata* populations, and this rare mussel now risks being extirpated from North Carolina (John Fridell, U.S. Fish and Wildlife Service, pers. comm.).

The recovery plan for *Lasmigona decorata* called for life history research as well as augmentation and reintroduction efforts (U.S. Fish and Wildlife Service 1996). Efforts to preserve and potentially augment populations through captive culture and propagation are dependent on our ability to select the most appropriate fish species to serve as fish hosts. Accordingly, we examined the ability of 27 species of fish, representing six families, from two North Carolina river basins to serve as potential hosts for captive *L. decorata* culture and propagation.

MATERIALS AND METHODS

Collection and holding of adult *Lasmigona decorata*

In August 2006, five *Lasmigona decorata* were collected from Duck Creek (Yadkin-Pee Dee River Basin) in Union County, North Carolina and immediately transported in a cooler wrapped in a wet cloth to the Table Rock Fish Hatchery near Morganton, North Carolina, where we maintained four troughs for culturing mussels. The hatchery was located on Irish Creek (Catawba River Basin), and the main water supply was a small (1.4 hectare) reservoir on the creek. Water pumped into the hatchery was mixed with water from a warmer, more nutrient-rich culture pond (0.4 hectare) and was piped continuously through the mussel culture trough. After exiting the trough, water flowed into a concrete raceway (4.5 m long $\times$ 1 m wide $\times$ 0.9 m deep), to allow settling of any larvae released by the mussels. The water was then pumped through a sand filter containing commercial filter media filtering down to 20–40 μ m (Aquatic Ecosystems, Inc., Apopka,

Florida). This prevented potential escape of mussels and their larvae not indigenous to the Catawba River Basin that were reared in the hatchery. In January 2007, two of the five adults were found to be gravid. One of those adults was transported in an aerated cooler of water to our propagation laboratory at North Carolina State University to be used as the source of glochidia for the initial host trial.

In February 2007, three adult *Lasmigona decorata* were collected in Sixmile Creek (Catawba River Basin) on the Union/Mecklenburg County line in North Carolina. One of those individuals was gravid and was maintained in the propagation laboratory for one week at $6 \pm 1^\circ\text{C}$ before being used as the source of glochidia for the second trial. Voucher representatives of both adults and glochidia of both the Duck Creek and Sixmile Creek stock were deposited in the North Carolina Museum of Natural Sciences, Raleigh, North Carolina.

Host fish trials

Yadkin-Pee Dee River Basin

On 18 January 2007, potential host fish representing 20 species (Table 1) were collected by seine and backpack electrofishing from Big Bear and Island Creeks in Stanly County, North Carolina and Irish Buffalo Creek in Cabarrus County, North Carolina (all in the Yadkin-Pee Dee River Basin). These streams were selected because they held few to no mussels, with no *Lasmigona decorata*, and naïve fish of a variety of species could be easily collected. Fish were transported in aerated coolers of stream water back to the propagation laboratory and maintained in various aquaria at $13 \pm 1^\circ\text{C}$. In January 2007, we extracted the glochidia from the single gravid adult from the Duck Creek stock by flushing the marsupium with a syringe filled with water from the mussel's tank. Mature glochidia were adhered to unfertilized eggs, forming conglomerates (Fig. 1). The size of these conglomerates made extraction somewhat difficult, and the repeated flushing necessary to complete the task caused some trauma to the gill tissue. This animal died within a week after glochidial extraction, and we believe it was a result of the gill trauma. We freed glochidia from conglomerates by repeatedly drawing the packets in and out of a plastic, 1-ml pipette. A small subsample (<100 individuals) of larvae were then tested for viability by exposing them to a salt solution (Zale and Neves 1982a). The brood was deemed viable when 100% of glochidia snapped shut in response to the salt solution. All fish were then placed together with the glochidia in approx. 12 liters of water for 30 minutes, and strong aeration was used to keep glochidia in suspension (approx. 2000 glochidia/liter). After infestation, fish were divided into different aquaria by species and maintained at $13 \pm 1^\circ\text{C}$. We used up to four aquarium replicates for some fish species when enough individuals of that species were available. We siphoned

Table 1. Yadkin-Pee Dee River Basin host trial for *Lasmigona decorata* from Duck Creek, Union County, North Carolina.

Common name (species)	No. of fish tested	No. of aquaria replicates	No. of juveniles produced	Mean percentage of glochidia transformed
Aphredoderidae				
Pirate perch (<i>Aphredoderus sayanus</i>)	5	3	1	0.9
Catostomidae				
Creek chubsucker (<i>Erimyzon oblongus</i>)	5	2	0	0.0
Centrarchidae				
Largemouth bass (<i>Micropterus salmoides</i>)	3	3	1	4.8
Redbreast sunfish (<i>Lepomis auritus</i>)	3	3	0	0.0
Green sunfish (<i>Lepomis cyanellus</i>)	3	3	0	0.0
Bluegill (<i>Lepomis macrochirus</i>)	15	3	185	23.3
Redear sunfish (<i>Lepomis microlophus</i>)	1	1	0	0.0
Cyprinidae				
Satinfin shiner (<i>Cyprinella analostana</i>)	1	1	6	54.5
Bluehead chub (<i>Nocomis leptoccephalus</i>)	4	2	67	73.7
Golden shiner (<i>Notemigonus crysoleucas</i>)	1	1	64	64.0
Whitemouth shiner (<i>Notropis alborus</i>)	9	3	2	16.7
Highfin shiner (<i>Notropis altipinnis</i>)	15	3	23	44.0
Redlip shiner (<i>Notropis chiliticus</i>)	7	2	1	3.1
Spottail shiner (<i>Notropis hudsonius</i>)	6	3	66	32.2
Ictaluridae				
Yellow bullhead (<i>Ameiurus natalis</i>)	1	1	2	10.5
Margined madtom (<i>Noturus insignis</i>)	6	2	0	0.0
Percidae				
Carolina darter (<i>Etheostoma collis</i>)	1	1	0	0.0
Fantail darter (<i>Etheostoma flabellare</i>)	12	4	1	0.1
Tessellated darter (<i>Etheostoma olmstedii</i>)	12	2	1	0.4
Piedmont darter (<i>Percina crassa</i>)	5	2	0	0.0

the bottom of each aquarium routinely (every 1-2 days) through a 150-mm mesh sieve to capture dead glochidia and transformed juvenile mussels for enumeration. Transformation success for each replicate was calculated by dividing the number of transformed juveniles by the sum of transformed juveniles and sloughed glochidia. Individuals were observed under a stereomicroscope and considered to be transformed if two adductor muscles were visible or if there was foot movement.

Catawba River Basin

On 1 March 2007, we collected potential host fish representing 17 species by seine and backpack electrofishing in the Twelvemile Creek system (Catawba River Basin) in Union County, North Carolina (Table 2). As with the Yadkin-Pee

Dee trial, these streams were selected because they contained few mussels. *Lasmigona decorata* did not occur in the stream, and naive fish of a variety of species could be easily collected. Fish were transported to the laboratory in aerated coolers of stream water and maintained in aquaria at 13 ± 1 °C. Because of the mortality caused by flushing of the marsupia in the initial trial, we elected to use chemical methods to induce release of glochidia in this trial. We immersed the mussel in 500 mg/L serotonin creatinine sulfate (Acros Organics, Geel, Belgium, 99% purity). The adult began releasing a few glochidia after 1 hour, but not nearly enough to conduct a host fish trial. After 3 hours, with some swelling of the mussel's foot tissue and few glochidia released, the mussel was removed from the serotonin and placed in chilled (13 °C) fresh water overnight. By the following morning (6 March

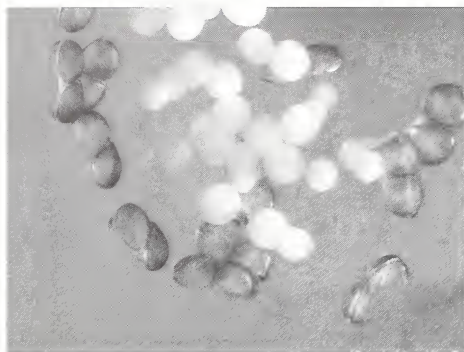


Figure 1. Viable glochidia of *Lasnigona decorata* adhered to undeveloped eggs.

2007), the entire brood had been released into the aquarium. Viability of the glochidia was again confirmed (100%) by exposing a small subsample of the brood to a saline solution as described above. Host fish and glochidia were then placed in 12 liters of water for 30 minutes and aerated vigorously to keep the glochidia in suspension (approx. 2500 glochidia/liter). Fish were then divided into separate aquaria by species and maintained at $13 \pm 1^\circ\text{C}$. We siphoned the bottom of each aquarium routinely (every 1–2 days) through a 150-mm mesh sieve to check for transformed juvenile mussels as described above for the Yadkin-Pee Dee trial. Because sloughed glochidia were not counted in this trial, we could not calculate the percentage of glochidia that transformed.

RESULTS

Description of glochidia

Glochidia were hooked, relatively large (mean = 305 ± 5 μm long $\times$ 256 ± 5 μm high), amber in color, and had the sub-triangular shape typical of the subfamily Anodontinae.

Table 2. Catawba River Basin host trial for *Lasnigona decorata* from Sixmile Creek, Union/Mecklenburg County, North Carolina.

Common name (species)	No. of fish tested	No. of aquaria replicates	No. of juveniles produced	Mean no. of juveniles per fish
Catostomidae				
White sucker (<i>Catostomus commersonii</i>)	2	2	0	0
Creek chubsucker (<i>Erimyzon oblongus</i>)	1	1	0	0
Striped jumprock (<i>Scartomyzon rupiscartes</i>)	1	1	0	0
Centrarchidae				
Wormouth (<i>Chaenobryttus gulosus</i>)	1	1	19	19
Redbreast sunfish (<i>Lepomis auritus</i>)	1	1	1	0.1
Green sunfish (<i>Lepomis cyanellus</i>)	1	1	7	7
Bluegill (<i>Lepomis macrochirus</i>)	2	2	0	0
Redear sunfish (<i>Lepomis microlophus</i>)	1	1	0	0
Cyprinidae				
Rosyside dace (<i>Clinostomus funduloides</i>)	3	1	37	12.3
Whitefin shiner (<i>Cyprinella nivea</i>)	2	1	29	14.5
Bluehead chub (<i>Nocomis leptcephalus</i>)	9	5	160	30.6
Golden shiner (<i>Notemigonus crysoleucas</i>)	32	5	541	20.7
Sandbar shiner (<i>Notropis scepticus</i>)	13	5	280	23.5
Creek chub (<i>Semotilus atromaculatus</i>)	1	1	41	41
Ictaluridae				
Flat bullhead (<i>Ameiurus platycephalus</i>)	2	3	1	0.3
Margined madtom (<i>Noturus insignis</i>)	2	1	0	0
Percidae				
Tessellated darter (<i>Etheostoma olmstedii</i>)	3	1	1	0.3

Most of the glochidia extracted by needle from the Yadkin-Pee Dee individual came out in semi-rectangular and relatively flat masses of eggs and glochidia (approx. 5–20 mm in size). These conglutinates were neutrally buoyant. The large glochidia were readily visible to the naked eye when viewed against the whitish, non-viable eggs to which they were attached (Fig. 1). The gravid adult from the Catawba River Basin that released its glochidia more naturally also produced conglutinates, but they were much smaller (<5 mm) and lacked a defined shape. When the released brood was observed the following morning, most glochidia were free of the unfertilized eggs, but were still somewhat stranded together in a heavy amount of mucous released by the adult.

Host fish trials

Yadkin Pee-Dee River Basin

A total of 420 transformed juveniles were collected from 13 of the 20 fish species tested; however, several of those species were poor hosts, yielding only one or two juveniles (Table 1). Five species of Cyprinidae, including the satfin shiner (*Cyprinella analostana*), bluehead chub (*Nocomis leptocephalus*), golden shiner (*Notemigonus crysoleucas*), highfin shiner (*Notropis altipinnis*), and the spottail shiner (*Notropis hudsonius*), acted as the most efficient hosts, with the percentage of glochidia transforming ranging from 32.2 to 73.7%. As a species, bluegill (*Lepomis macrochirus*) produced the most juveniles, but this production was attributed to the use of 15 individuals of this species. Two replicates containing individual bluegill produced only four (13.3% transformation) and six (33.3% transformation) metamorphosed individuals, respectively. Another aquarium of bluegill containing 13 individuals produced 175 juveniles. When individual fish from this tank were examined during the encystment period, several glochidia were readily visible on the gills and fins of some fish while other fish appeared to have no larvae attached. The length of time attached to all fish ranged from 22 to 44 days (Table 3).

Catawba River Basin

Some pedal swelling and gaping was observed when the adult mussel was exposed to serotonin, but this completely subsided within 24 hours once the animal was removed from the exposure. No other ill effects were noted, and the animal was maintained at the hatchery for six months after exposure. The animal was observed to have good adductor muscle strength and burrowing ability during this time and was presumed healthy.

Table 3. Time to complete transformation for each species of fish tested in trial 1 (Yadkin-Pee Dee River Basin).

Common name (species)	N	Median transformation time with quartiles (days)	Range (days)
Bluegill (<i>Lepomis macrochirus</i>)	185	27 (24, 30)	22–44
Satfin shiner (<i>Cyprinella analostana</i>)	6	35 (30, 37)	29–41
Bluehead chub (<i>Nocomis leptocephalus</i>)	67	34 (34, 36)	24–44
Golden shiner (<i>Notemigonus crysoleucas</i>)	64	36 (34, 38)	27–44
Highfin shiner (<i>Notropis altipinnis</i>)	23	30 (29, 35)	24–41
Spotfin shiner (<i>Notropis hudsonius</i>)	66	29 (27, 30)	22–41

A total of 1,117 juveniles were collected from 11 of the 17 fish species tested; however, as with the Yadkin-Pee Dee trial, several species of Cyprinidae supported the greatest percentages of transformation (Table 2). Rosyside dace (*Clinostomus funduloides*), whitefin shiners (*Cyprinella nivea*), sandbar shiners (*Notropis septicus*), and the creek chub (*Semotilus atromaculatus*) represented new hosts not tested in the Yadkin-Pee Dee trial. Bluehead chubs and golden shiners served as hosts again as in the initial trial. A smaller number of juveniles transformed on the green sunfish (*Lepomis cyanellus*) and warmouth (*Chaenobryttus gulosus*). Unlike in the Yadkin-Pee Dee Trial, no juveniles transformed on the two bluegills tested.

DISCUSSION

Prior to this study, very little was known about the life history of this species (Bogan 2002). One gravid individual had been observed in October (Bogan 2002). Since we found the species gravid in January and late February, it is apparently bradytictic, spawning in late summer or fall, and releasing glochidia in late winter or spring of the following year. Determination of the required host fish of *Lasmigona decorata* will allow propagation and captive culture of this rare species to move forward for conservation purposes. We can only speculate as to the reason why the glochidia appeared to be packaged differently between the two trials. Both individuals yielded glochidia attached to non-viable eggs, but the individual that was chemically induced to release produced much smaller conglutinates. We also found more glochidia unattached to eggs released by the mussel several hours after the serotonin exposure. Because much of that release occurred overnight, we do not know what percentage of the released glochidia were originally attached to eggs, but came detached before they were observed. It may be that both individuals naturally packaged their glochidia with non-viable eggs similarly within the marsupium, but the mussel exposed

to serotonin simply broke the mass into smaller pieces than those that were manually removed by syringe. None of the conglomerates had a well-defined shape, and they may have broken up differently based on how they left the mussel. Aside from possible variation between individuals or between basins, another possible explanation could be differences in fertilization success between the two females. Because of our need to use as many of these rare glochidia as possible as quickly as possible on the host fish, we did not attempt to quantify the total number of glochidia produced or the ratio of viable glochidia to non-viable eggs.

Lasnigona decorata juveniles were successfully transformed from glochidia attached to multiple fish species. Between the two trials, a total of nine species from the Cyprinidae family served as efficient hosts. The redbelly shiner (*Notropis chilinticus*) and whitemouth shiner (*Notropis alborus*) were the only cyprinid species considered poor hosts due to the small proportion of larvae that completed a metamorphosis on those species. A small number of juveniles were recovered from the highfin shiner (*Notropis altipinnis*), but only a small number actually attached—likely due to the small size of the individuals (<40 mm) used in the trial. However, 44% of those that attached successfully transformed. In the Centrarchidae, the bluegill served as a host in the Yadkin-Pee Dee trial but yielded no metamorphosed juveniles when tested with the Catawba mussel, and the green sunfish successfully supported the metamorphosis of seven individuals in the Catawba trial but failed as a potential host in the Yadkin-Pee Dee trial. Because only a small number of bluegill were used in the Catawba trial, and only one green sunfish was used in each trial, the reason for the variation in their ability to serve as hosts is not known. Our results, however, may indicate differences in host suitability between two river basins, which has been previously demonstrated (Rogers *et al.* 2001, Bigham 2002, Eckert 2003). Because these two river basins drain separately to the Atlantic Ocean, the fish tested in this study were genetically isolated and may have different susceptibilities to infection by a given mussel species. We were limited to a single female mussel from each basin because *L. decorata* is such a rare species, and the lack of replication prevented us from making a definitive conclusion. In addition, the glochidia extraction method may have accounted for a portion of the variability between the two trials. To accurately determine host fish requirements, future studies should strive to use fish from the same river basin as the mussel being tested and use multiple brooding females if possible.

Several other mussel species in the subfamily Anodontinae are relative host generalists, using fish from multiple families (Watters *et al.* 1998, McGill *et al.* 2002, Van Snik Gray *et al.* 2002) as hosts. While *Lasnigona decorata* did transform on three species of Centrarchidae, cyprinids were far more effective at facilitating transformation. The heel-

splitter is typically found in deep runs as well as pools, and occurs in a variety of substrates from soft mud to clean sandy-gravel and even occurs between large boulders within pools (Keferl and Shelley 1988, and the authors' pers. obs.). These habitat preferences should overlap considerably with the preferences of both the centrarchids and the variety of cyprinids we identified as viable hosts. Though some identified hosts, such as the bluehead chub, are more benthic in nature and may come in closer contact with the mussel, the neutrally buoyant conglomerates produced by this species should also provide dispersal of glochidia to fish up in the water column. We believe these small, white packages of glochidia would likely be attractive as a potential food source to many cyprinid species, but attempts by the fish to eat them would yield an infestation of glochidia rather than a true meal.

The bluehead chubs and golden shiners appeared to be the best choices as fish hosts for captive propagation. The bluehead chub yielded the largest proportion of glochidia undergoing successful metamorphosis (73.7%), and this species is widespread, abundant and relatively easy to capture by electrofishing or seining. Golden shiners, the second most efficient host (64.0% transformation), produced large numbers of juveniles and can be easily purchased from a local bait shop in large numbers at a relatively low price. However, baitfish used for captive propagation should preferably be obtained from baitfish suppliers who adhere to recommended health certification guidelines.

Extraction of glochidia from conglomerate-producing mussel species can be difficult, and flushing of the gills requires additional effort compared to species whose glochidia are not connected in packets. Relatively large openings must be made in gill tissue to extract large conglomerates from the marsupia, and this can be damaging to the gill tissue and even fatal, as in our study. Allowing a gravid mussel to release glochidia on its own often results in release of a small percentage of the brood over several days or weeks (Eads, pers. obs.), making propagation more difficult and well-controlled host determination experiments almost impossible. Therefore, we elected to expose the gravid *Lasnigona decorata* in our second trial (Catawba Basin) to serotonin, a neurotransmitter known to affect many aspects of bivalve reproduction including induction of parturition (abortion of immature glochidia; Fong and Warner 1995) and glochidial release (Dimock and Strube 1994). Additionally, selective serotonin reuptake inhibitor (SSRI) drugs, which increase serotonin neurotransmission, have been shown to cause parturition or glochidia release in the fingernail clam *Sphaerium striatinum* (Lamarck, 1818) (Fong *et al.* 1998), the zebra mussel *Dreissena polymorpha* (Pallas, 1769) (Fong *et al.* 2003), and the freshwater mussel *Anodonta cygnea* (Linnaeus, 1758) (Cunha and Machado 2001).

This study does not provide a thorough direct comparison between physical and chemical glochidia extraction methods, but it does identify an area of potential research for advancement in propagation methodologies. In our work, the use of serotonin was effective in allowing the collection of all glochidia in the brood apparently without lasting harm to the adult mussel. Glochidia also retained their viability, which was documented by a 100% response to a salt solution and their transformation on host fish. There were short-term physical effects observed in the mussel. We observed a strong increase in volume of the foot in *Lasmigona decorata* during early stages of exposure to serotonin, consistent with the response in *Anodonta cygnea* reported by Cunha and Machado (2001), who attributed the increase in foot size to relaxation of foot muscles and the resulting favorable conditions for uptake and storage of water. The *L. decorata* used in our trial recovered (*i.e.*, foot returned to pre-serotonin exposure size) within 24 hours of placement in clean water. Though our work with serotonin was successful, we suggest additional experimentation with varied concentrations and immersion times to optimize induction of glochidia release with minimal serotonin exposure. Additional studies are needed to determine the effects of serotonin on glochidial viability, gill respiration, the potential for future reproduction, and the long-term overall health of gravid females. As the use of serotonin or other similar compounds is refined, it may prove a useful tool in the simple and safe extraction of glochidia for propagation of freshwater mussels.

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